A METHOD FOR RECORDING AND ANALYZING EEGs AND VERs FROM RATS UNDER HYPERBARIC CONDITIONS

by

Steven H. Ferris, Ph.D.
and
Raymond T. Bartus, Ph.D.

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Transmitted by:

Jo Ann S. Kinney

Jo Ann S. Kinney, Ph.D.
Head, Vision Branch

Reviewed and Approved by:

Charles F. Gell

Charles F. Gell, M.D., D.Sc. (Med)
SCIENTIFIC DIRECTOR
NavSubMedRschLab

Approved and Released by:

R. L. Sphar

R. L. Sphar, CDR, MC USN
OFFICER IN CHARGE
NavSubMedRschLab

SUMMARY PAGE

THE PROBLEM

To develop a technique for recording electroencephalograms and visual evoked cortical potentials from rats during hyperbaric exposure.

FINDINGS

A chronic electrode implantation technique was developed which is considered reliable and durable. EEGs and VERs were obtained from implanted rats exposed to high pressure.

APPLICATION

The methodology which has been developed is well-suited for studying the neurophysiological changes which occur in animals exposed to hyperbaric environments.

ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Bureau of Medicine and Surgery Research Unit MF51.524.004-9015DA5G. The present report is Number 5 on this work unit. It was submitted on 4 June 1973, approved for publication on 12 July 1973 and designated as NavSubMedRschLab Report No. 747.

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ABSTRACT

A technique is described for recording electroencephalograms (EEGs) and visually evoked cortical responses (VERs) from rats during hyperbaric exposure. A reliable and durable chronic electrode implantation procedure is used in which miniature, self-tapping stainless steel screws serve a dual role as cortical electrodes and as anchors for a miniature connecting socket. Sample data are presented which were obtained from implanted rats during exposure to hyperbaric air. The techniques developed are well-suited for studying the neurophysiological effects of hyperbaric environments.

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INTRODUCTION

In order to study the neurophysiological changes caused by exposure to high pressure, a simple, reliable procedure was developed for recording the electrical activity of the brain. This procedure involves the chronic implantation of monopolar electrodes over the rat's visual cortex, permitting the recording of ongoing cortical activity (EEGs) as well as the specific neural responses evoked by visual stimulation (VERs). The following report summarizes the final technique developed for the chronic implantation of the electrodes, as well as the methods used to record and analyze the EEGs and VERs.

METHOD

Chronic Electrode Implantation

Standard aseptic surgical procedures are maintained throughout the procedure. The rats are anesthetized with sodium pentobaritol and mounted onto a standard stereotaxic instrument. A single incision (approximately 3 cm. long) is made along the midline, and the dorsal surface of the skull is exposed and rubbed clean. Three small holes marking the electrode sites are then hand-bored through the skull, positioned according to skull landmarks. The hole for the active electrode is placed over the rat's primary visual cortex (3 mm lateral and 2 mm anterior to the lambda), and the reference and ground electrode holes are placed near the ipsilateral and contralateral frontal sinuses, respectively (approximately 3 mm lateral and 5 mm anterior to the bregma). Three miniature, self-tapping stainless steel screws (size 00, 1/8 in. long)[1] are screwed into the skull holes, providing tightly secured epidural electrodes (see Fig. 1). The small size and self-tapping feature of these screws provides a very strong attachment to the thin skull of the rat, thus serving the additional function of anchoring the entire assembly to the rat.

The connecting socket, which is mounted on the dorsal surface of the skull, is assembled prior to surgery. A six-hole length is cut from an Amphenol miniature strip connector.[2] Three lengths of teflon-coated stainless steel wire[3] are crimped to Amphenol Mini-Tac receptacles.[4] The receptacles are then inserted into holes 2, 3 and 5 of the strip connector. After the screw electrodes are mounted in the skull, the bared ends of the wire are wrapped tightly around the screws, thus securing the socket assembly in place (see Fig. 2). The screws and socket assembly are then embedded in a mound

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1. *Available from J. I. Morris Co., Southbridge, Mass. 01550.*
2. *Amphenol part No. 221-3580, available from Amphenol Industrial Div., 1830 South 54th Ave., Chicago, Ill. 60650.*
3. *Size 316SS10T, available from Medwire Corp., Mount Vernon, N.Y. 10553.*
4. *Amphenol part No. 221-760.*

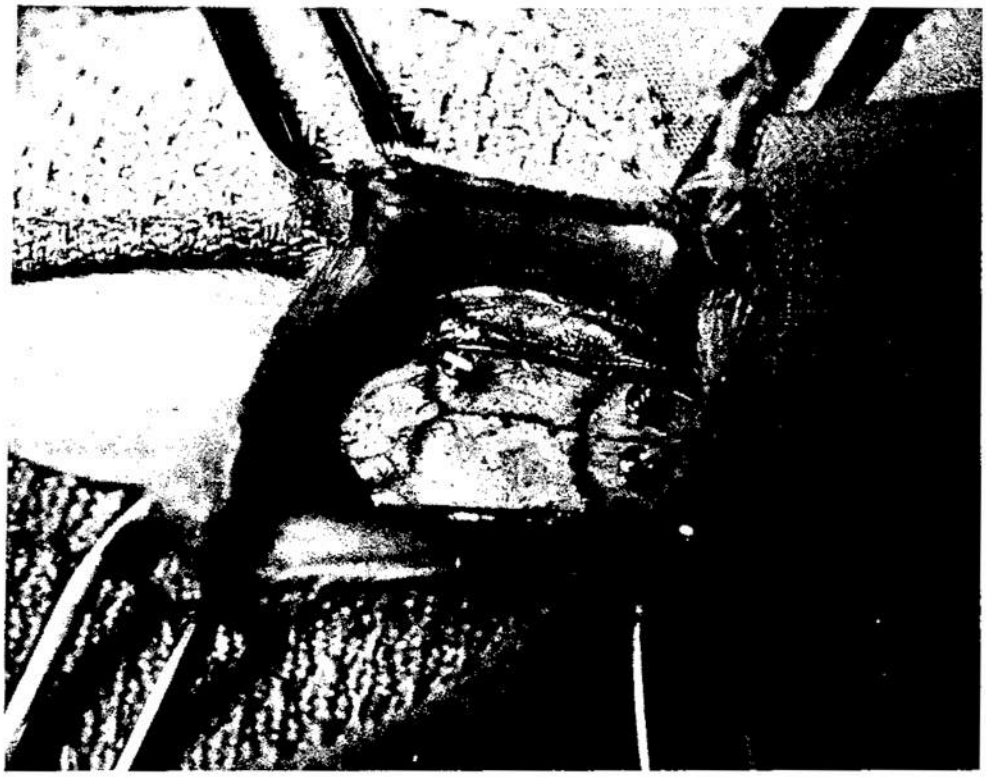

Fig. 1. Exposed rat skull, showing bone landmarks and location of screw electrodes.

of dental acrylic,[5] securing the screws, assembly, and skull as a single mass. After the acrylic hardens, the incision is sutured around the base of the mound, providing a compact and rigid pedestal. A fully recovered, implanted rat is shown in Figure 3.

To facilitate daily recording of the implanted animals, a special plug was designed that could be quickly inserted into the implanted socket without easily pulling out. The plug is assembled by crimping low-noise cable (from worn Grass scalp electrodes) to three Amphenol Mini-Tac pins,[6] and inserting the pins into holes 3, 4, and 6 of an eight-hole Amphenol miniature strip connector.[7] Special guide pins, made by filing No. 20, 1/2 in. brads to an appropriate length and thickness, are inserted into holes 2 and 7 of the plug. These pins fit tightly into holes 1 and 6 of the implanted socket, thus serving to guide and lock the plug in place. An eight-hole Amphenol strain-relief strip[8] is fastened to the plug with two locking pins[9] inserted into holes 1 and 8. The three plug cables are braided to reduce noise, and additional strain-relief is

5. *"Grip" cement, available from L. D. Caulk Co., Milford, Del. 19963.*

6. *Amphenol part No. 221-3680.*
7. *Amphenol part No. 221-759.*
8. *Amphenol part No. 221-9380.*
9. *Amphenol part No. 221-763.*

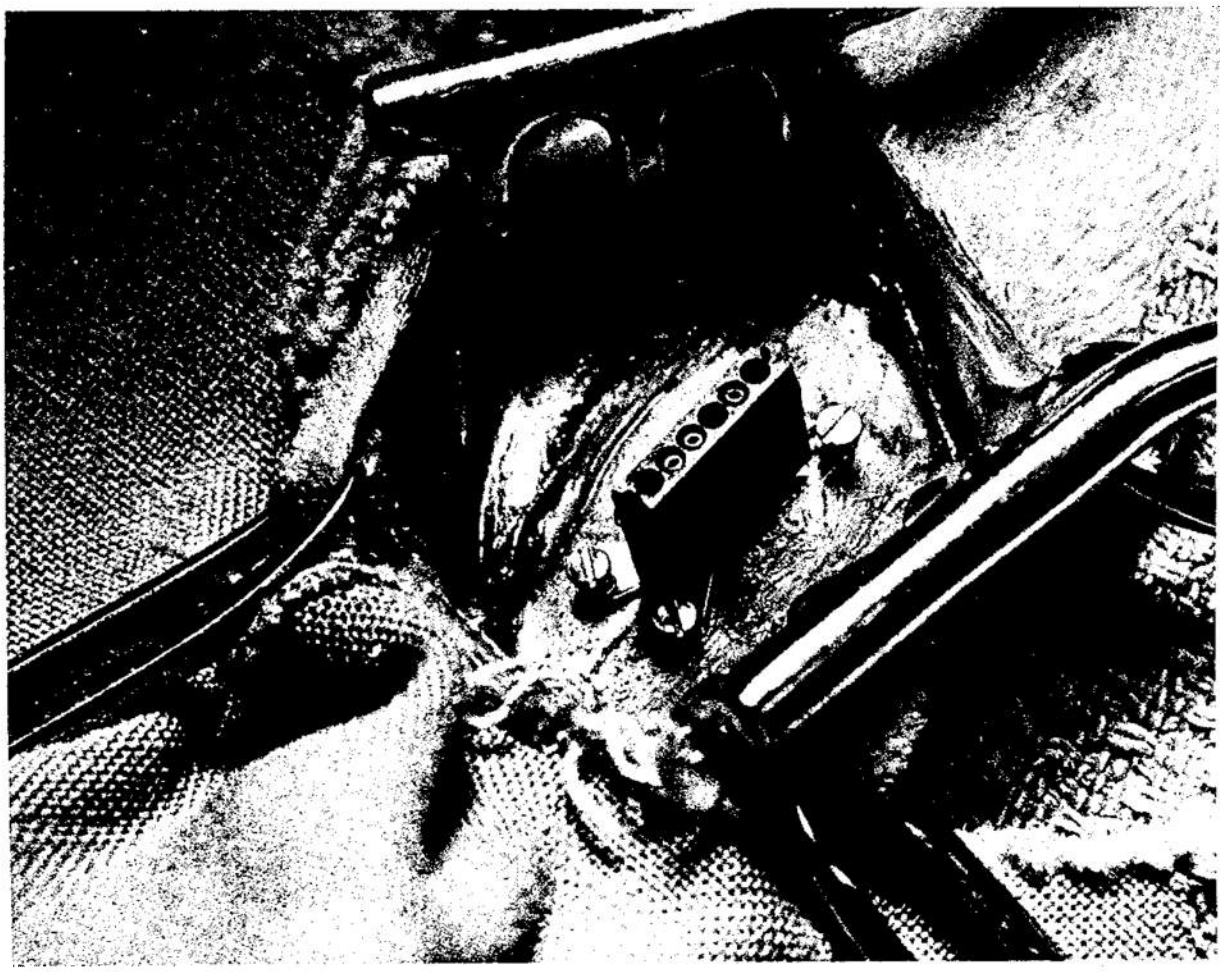

Fig. 2. Connecting socket mounted on the rat skull. Wires from the three electrical receptacles are wrapped tightly around the screw electrodes.

provided by wrapping the plug assembly. and part of the cable with cloth tape.

Recording Technique

EEGs and VERs under hyperbaric conditions are obtained using a small Bethlehem chamber capable of reaching 1000 PSI. Electrical penetration is by means of a 16 conductor cable attached to a plug on the inside, and a terminal strip on the outside. Two rats at a time are placed in the chamber. They are held in separate compartments of a specially designed, plexiglas restraining box (see Fig. 4). Although the rats will resist being placed in the restrainer from above, they readily walk in by themselves through the hatch at each end of the box. The cables from the two plugs used for connecting the rats' electrodes to the amplifiers are permanently attached to a single receptacle which connects to the plug inside the chamber.

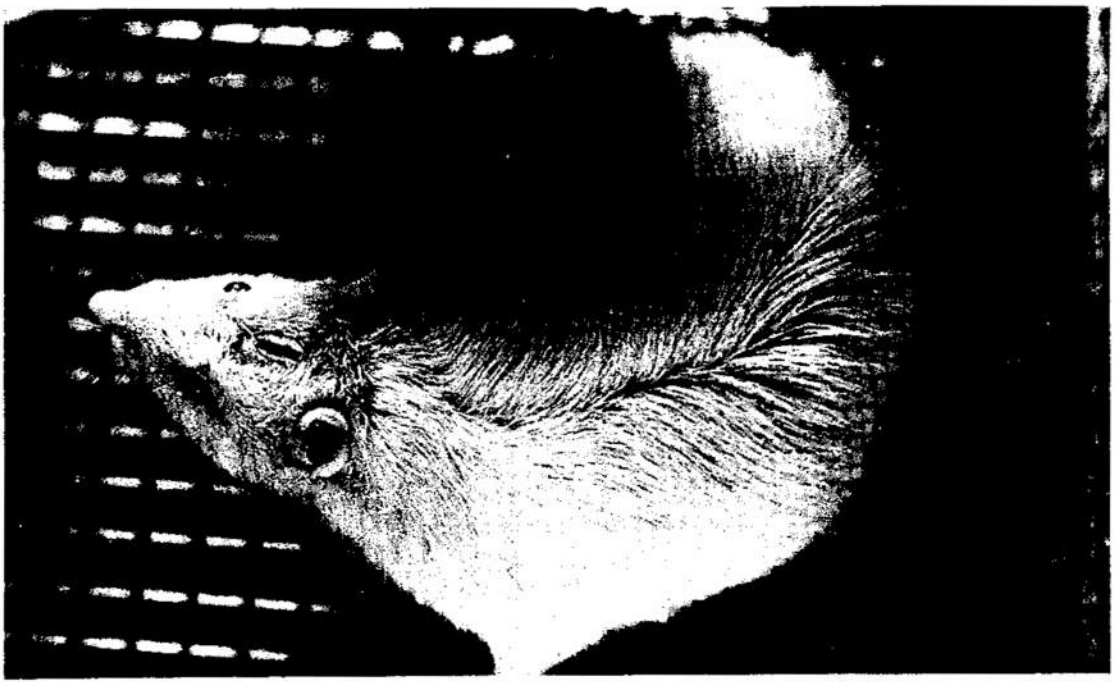

Fig. 3. A fully recovered implanted rat.

The EEG from each rat is amplified by a separate Grass P511 preamplifier and recorded on FM tape (Hewlett-Packard Model HP 3000 recorder). A frequency analysis (power spectral density) of the recorded EEG is later obtained using a Federal Scientific Co. spectrum analyzer (Model UA-10A) and spectrum averager (Model 1010). The visual stimulation for VERs is provided by flashing a Grass PS-2 photostimulator directly into the porthole of the chamber. The amplified EEG of each rat is connected on-line to separate channels of a Computer of Average Transients (Technical Measurement Corp.) which enhances that portion of the ongoing EEG that is time-locked to the light flashes. Paper records of the averaged visual evoked responses are obtained with an X, Y recorder.

RESULTS AND DISCUSSION

A typical EEG recording from an unanesthetized, chronically implanted rat is shown in Fig. 5. It is apparent that noise and movement artifacts are negligible with this implantation and recording technique. The traces below the EEG are frequency analyses for separate 4 sec. EEG epochs. Averaged frequency spectra for 16 epochs are shown in Fig. 6. These results are for three different rats, each recorded three times at weekly intervals. The high degree of intra-rat reliability and inter-rat similarity is quite apparent. The peaks in the 5-9 Hz range are characteristic of the theta activity commonly observed in rats.

VERs obtained immediately after the EEG results illustrated in Fig. 6 are

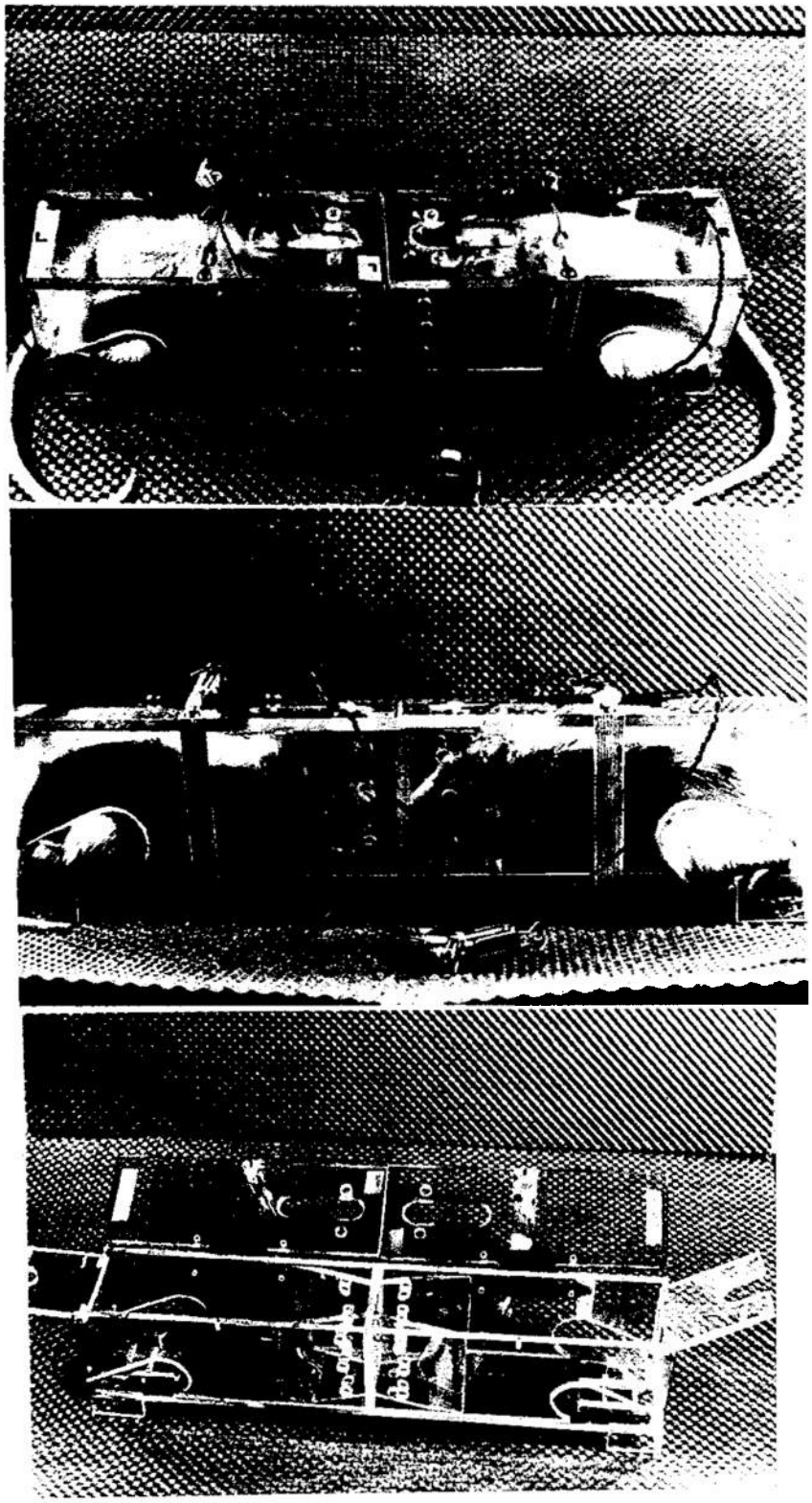

Fig. 4. Two implanted rats, plugged in and in place in the specially designed restraining box.

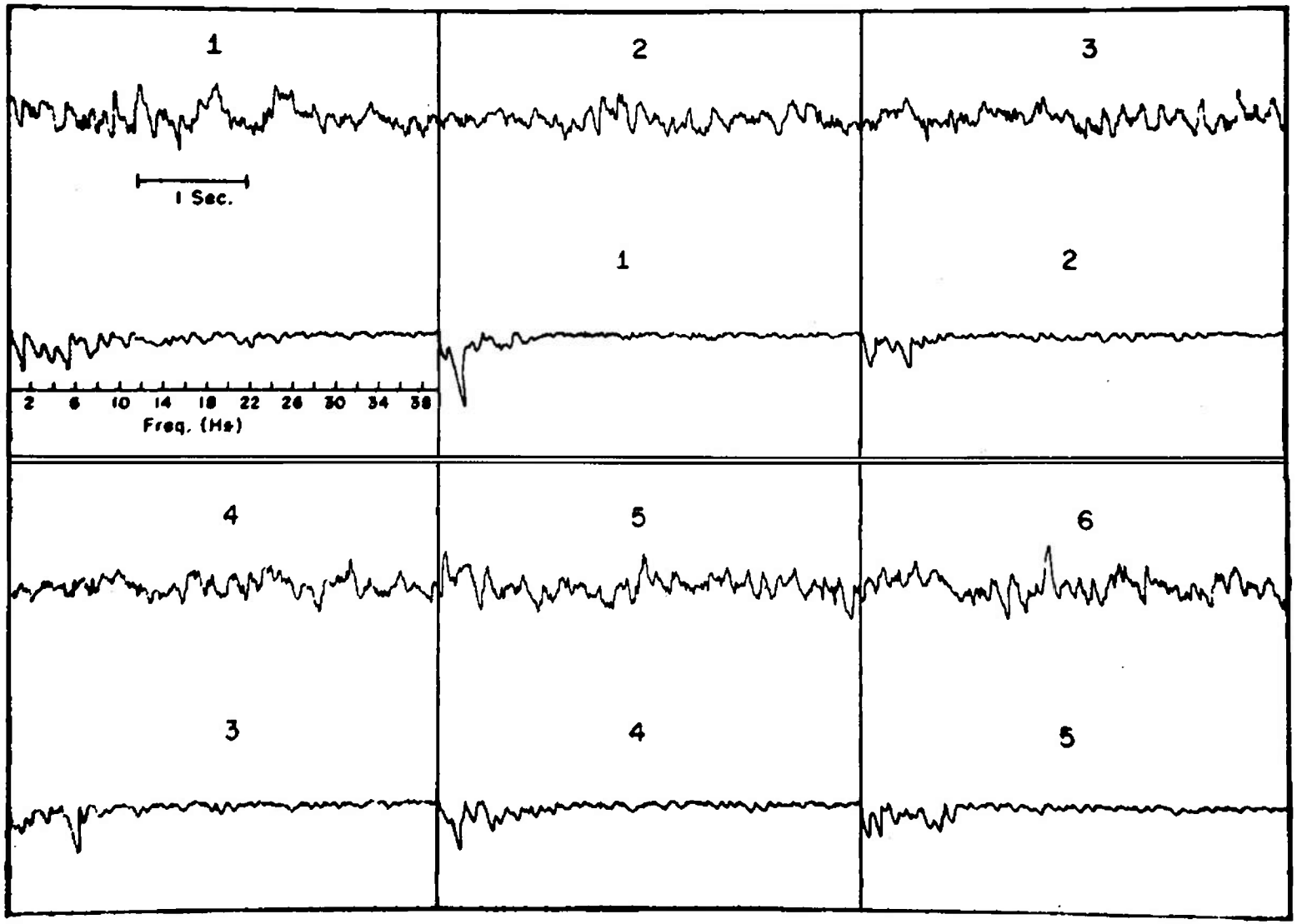

Fig. 5. Sample EEG recorded from an implanted rat. The traces below the EEG are frequency analyses (0-40 Hz.) for separate 4 sec. EEG epochs. Each analysis is for the preceding EEG epoch.

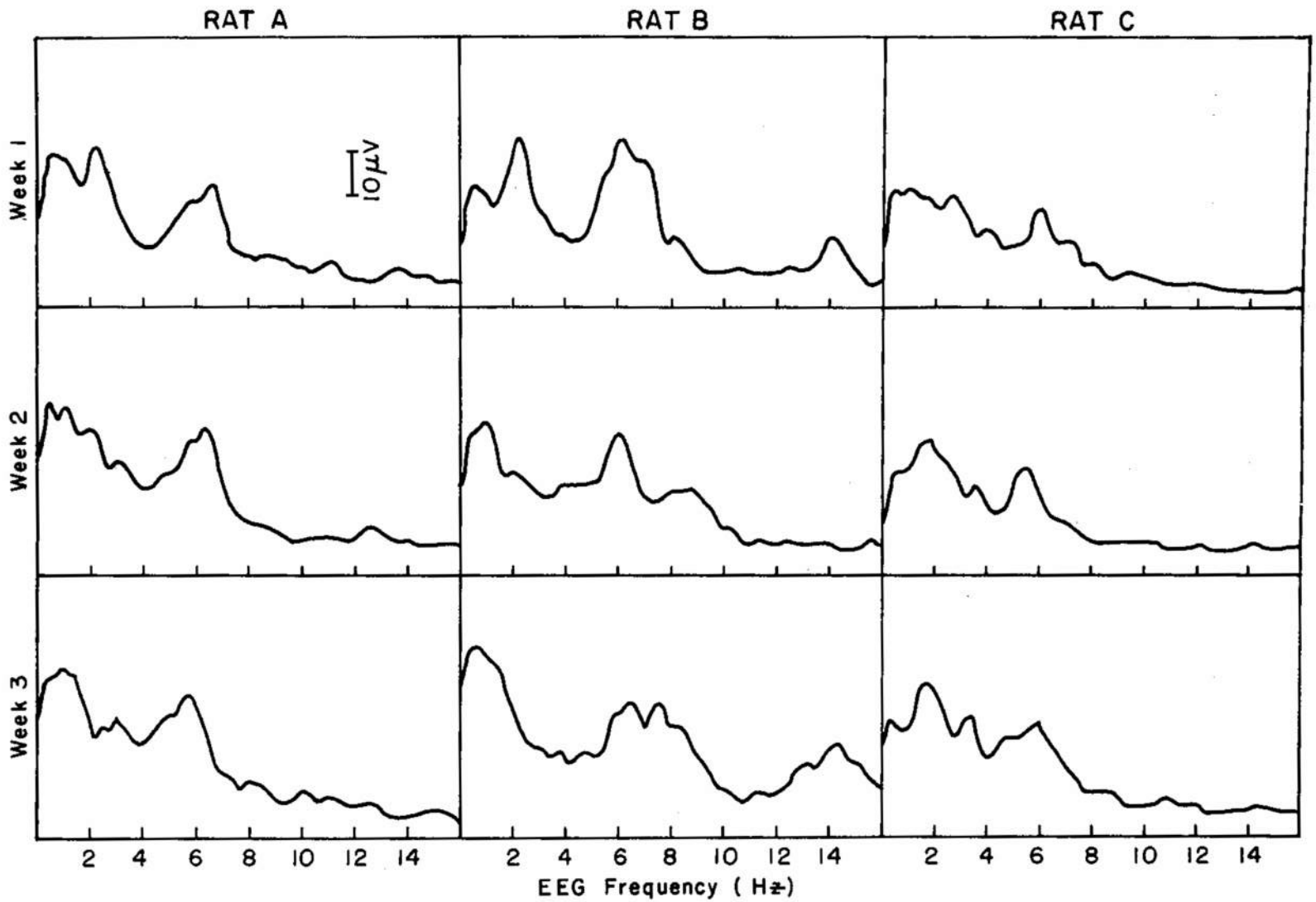

Fig. 6. Averaged frequency spectra (for sixteen 4 sec. EEG epochs) for three rats, each recorded on three successive weeks.

shown in Fig. 7. The reliability of the implantation and recording techn.que is again apparent. Although amplitude differences commonly occur, different rats have similar major components, and a particular rat shows very little change from week to week. Typical VERs obtained during a bounce dive to 300 ft, using compressed air, are illustrated in Fig. 8. The characteristic components are labeled a-f on the predive record. Each VER is quantified by measuring the major peak to peak deflections and comparing across conditions. For the data illustrated, there was a 41 percent decrease in amplitude at 300 ft (averaged across components) as compared with the average of the predive and postdive records.

The methods described in the present report are ideally suited for studying the electrophysiological changes produced by hyperbaric conditions. This chronic implantation technique has been tested and found to be reliable and durable, providing consistent recordings for six months or more in most cases. The technique may be extended to include depth electrodes placed in appropriate sub-cortical areas and may also be used with task-trained animals to simultaneously assess the behavioral and neurological effects of hyperbaric environments. Finally, with slight modification, the technique discussed in the present report may be adapted for use with larger animals, such as cats and monkeys.

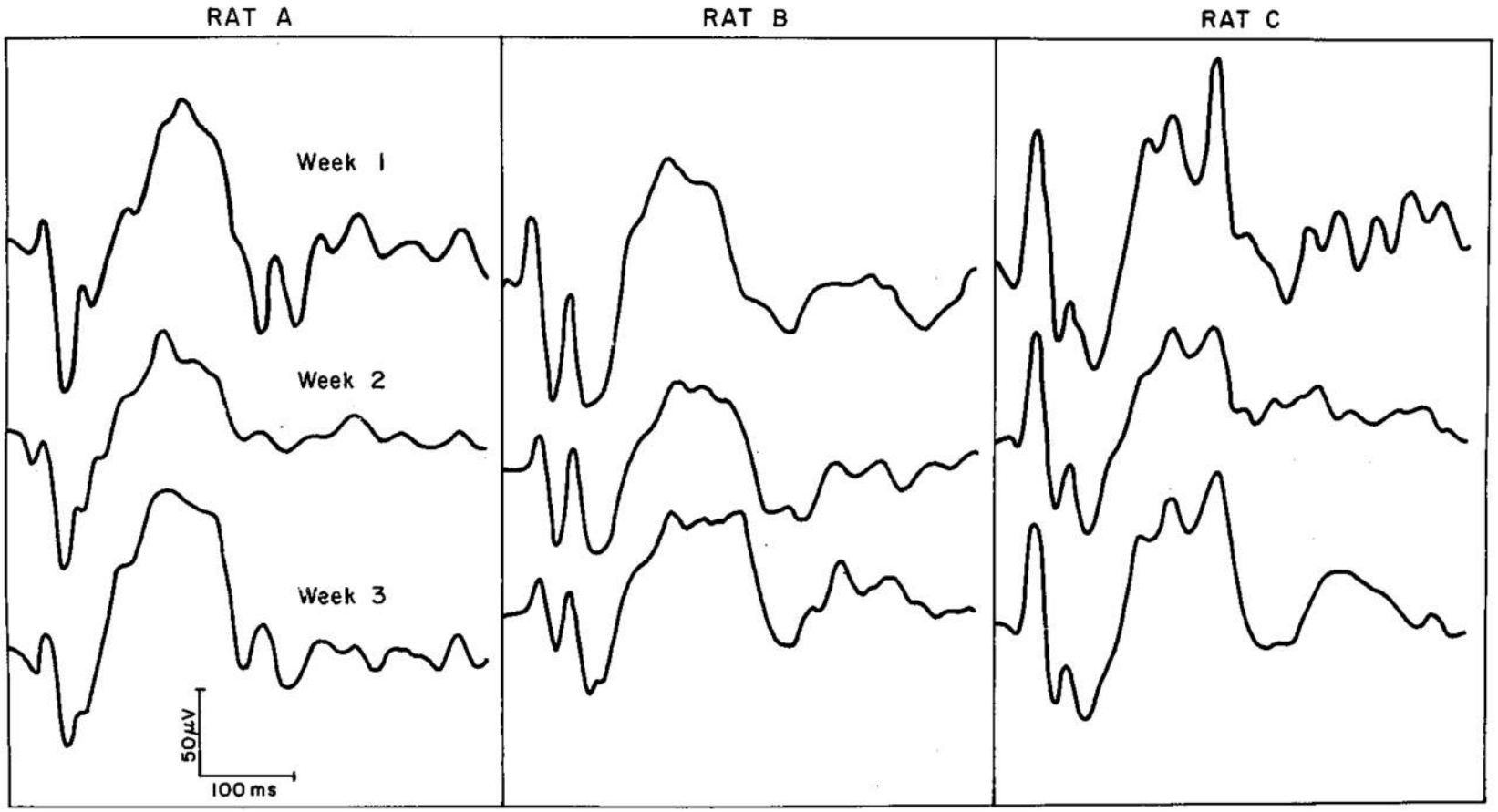

Fig. 7. VERs obtained from three rats, each recorded on three successive weeks. These records, which represent the average of 100 one sec. sweeps, were obtained immediately after the EEG analyzed in Fig. 5 was recorded.

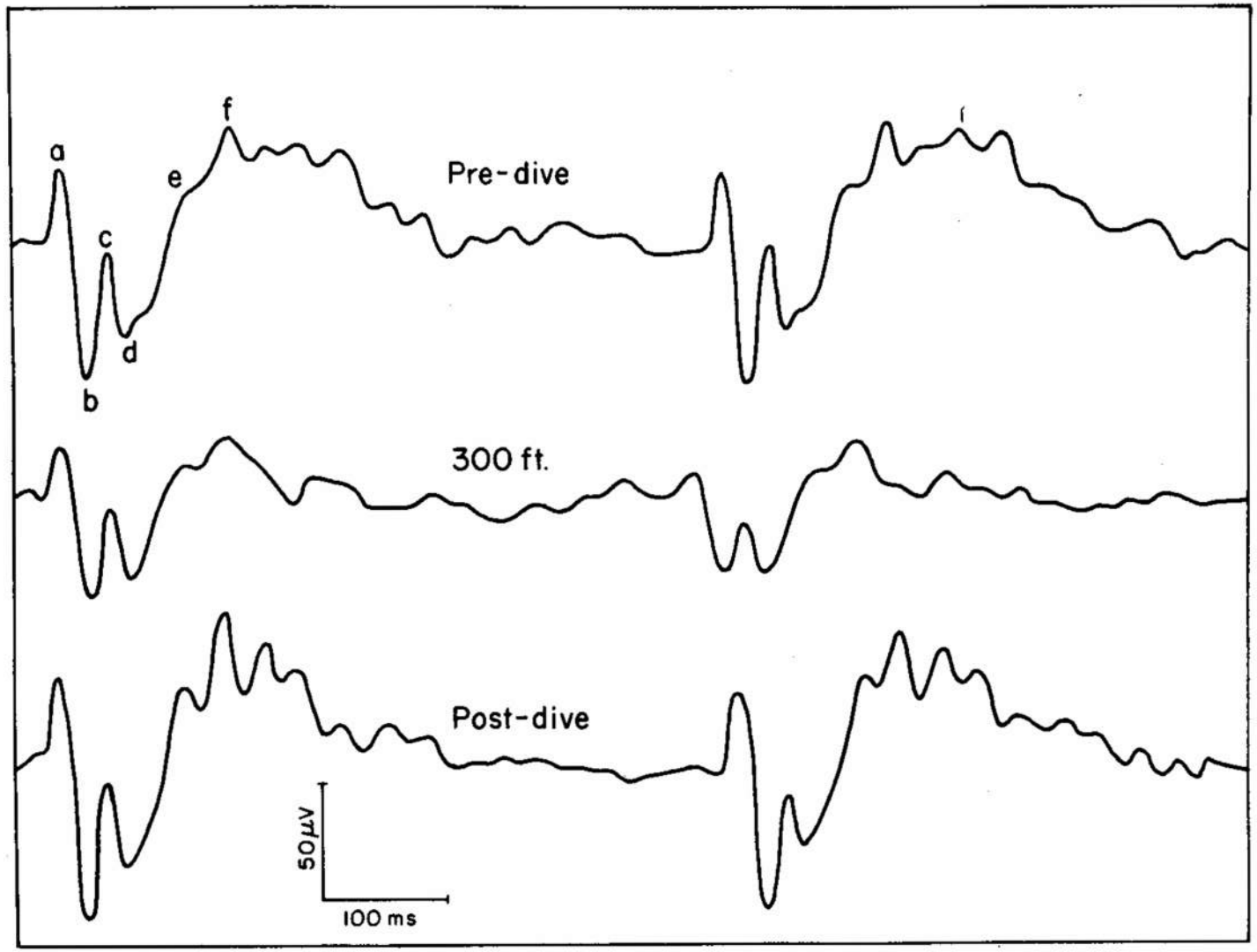

Fig. 8. VERs obtained during a bounce dive to 300 ft using compressed air. The flash frequency was 2 Hz. and 100 one sec. sweeps were averaged.

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